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Accounting for Environmental DNA When Monitoring Fresh Surface Waters for *E. coli* and Enterococci by QPCR

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ABSTRACT

Molecular methods such as quantitative polymerase chain reaction (QPCR) have been examined for their utility in monitoring water quality indicators. Elevated enterococcal cell equivalents (CE) demonstrated correlation to human health effects at bathing beaches in proximity to a sanitary source. However, similar epidemiological studies have not been conducted at freshwater beaches dominated by non-human, non-point sources of contamination. Environmental DNA contributions from dead cells may alter water quality results in the absence of health risk. The purpose of this study was to determine if CE were correlated to currently approved water quality indicators (actual values and resulting regulatory action) and when a disparity existed whether or not it could be predicted by selected environmental factors. Samples collected at two bathing beaches and three control sites in Racine, WI were monitored for *E. coli* and enterococci (IDEXX Colilert™, mEI agar, and QPCR equivalent methods). Environmental data was collected using the EPA routine beach survey water samples, wave height, antecedent precipitation, and flow rate (river sites). Paired cell counts and CE data was recorded daily (n = 12, 2007; n = 45, 2008). When correlation fell below 50% (R² < 0.5) it was frequently predictable (R² sites). At beaches, disparity in cell count vs. CE could be predicted by 24 hr rainfall (< 1.72 cm) and wave height (> 30.48 cm). River sites were more variable, one correlating to precipitation (> 1 cm), one to flow rate, and one not correlating to assessed environmental factors. A corrective factor was developed (AVG [CE-cell count] seasonally) and applied on days when site specific environmental conditions were met, i.e. rainfall > 1.72 cm then, CE Corrected = CE initial – (AVG [CE-MPN] seasonally). Application of this factor improved beach status prediction from 40 to 98% (e.g. *E. coli* and enterococci). *E. coli* and enterococci were correlated (R² = 0.67). Accounting for DNA contributions from dead cells may be necessary at non-point source dominated surface waters prior to developing and implementing regulatory standards based on molecular methods.

INTRODUCTION

Real-time testing of surface water quality would be beneficial to public health agencies. Rapid testing methodologies, such as quantitative real-time polymerase chain reaction (QPCR), are intended to shorten the period between sampling and publicly available results, with the goal of having same day water quality information. To date, no QPCR method is currently approved for monitoring, however it is clear that interest in the same has grown following the published language in the Beach Protection Act of 2000 (HR 2437 and S 2464) and recently introduced Clean Coastal Environment and Public Health Act of 2009 (HR. 2437 and S. 2464) and recently introduced Clean Coastal Environment and Public Health Act of 2009 (HR. 2437 and S. 2464). Previously developed methods for QPCR, using the indicator organisms (*E. coli* and enterococci), have created the potential for a viable real-time assessment tool. However, for these methods to utilized successfully, additional communications need to be taken into account such as the ability of the method to distinguish recent contamination events (which may result in adverse health outcomes in exposed individuals) from the re-suspension of non-viable organisms from non-point sources such as farmfield runoffs and sediments. Substantial amounts of free environmental DNA or DNA associated with dead cells, exist it may hamper the ability of monitoring agencies to detect true elevations in bacterial indicator organisms at sites influenced by point source or co-ripped pollutant sources when using QPCR as the detection method. Once received, real-time monitoring capability would enhance the efforts of municipal city departments in identifying and responding to incidences of potential loading in a timely and effective manner, in particular in response to water quality advisories at recreational bathing beaches.

METHODS

Surface water samples from Lake Michigan (North and Zoo Beaches) were collected into sterile Whirl-Field™ bags, composited as per Kinzelman et al. 2006 (4 transects/beach) and analyzed for viable cell counts using either IDEXX Colilert™ (E. coli) or mEI agar (USEPA Method 1605). Enterococci and/or by QPCR equivalent methods (Bacillus CSR/Cepheid QMx™ (as per Blackwood et al. 2008)) for E. coli and TaqMan® for Enterococci (as per Haugland et al. 2005) to quantify E. coli and Enterococci and express as cell equivalents (CE) (Fig. 1). River site samples were collected over three years from overpassing bridges at these locations and analyzed in the same manner as the Lake Michigan samples. Comparisons were made between QPCR duplicate CE values and MPN/RMP at each beach or river site to determine the degree of difference between table cell counts and total DNA (as CE) with respect to both actual organisms numbers and resulting regulatory action (Fig. 2). When a disparity existed the following factor was applied: CE Corrected = CE initial – (AVG [CE-MPN] seasonally) (Figs. 3 and 4). Table 2 lists the relationships between measured cell count and corrected CE.

RESULTS

**Table 1.** E. coli concentrations at North and Zoo Beaches based on daily composite samples (2008). QPCR prediction = range of CE values from duplicate analyses corrected for ambient background DNA. Table 1 shows a range of sample means and displayed medians where E. coli QPCR prediction did not match regulatory target or highlight in yellow.

**Table 2.** Enterococci concentrations at North and Zoo Beaches based on daily composite samples (2008). QPCR prediction = range of CE values from duplicate analyses corrected for ambient background DNA. Table 2 shows a range of sample means and displayed medians where Enterococci QPCR prediction did not match regulatory target or highlight in yellow.

**Figure 1.** A factor for QPCR (CE-sample collection; [b] seaweed exclusion; [c] enteric mix + target; [d] real-time PCR; [e] continuous monitoring in the field.)

**Figure 2.** Uncorrected E. coli versus enterococci CE, North and Zoo Beaches combined data (2008). y = 0.6069x + 1.5719 R² = 0.26

**Figure 3.** Uncorrected E. coli versus enterococci CE Summer 08. y = 0.6884x + 0.4158 R² = 0.618

**Figure 4.** Adjusted 2.50 CE (uncorrected E. coli) and enterococci CE (corrected for non-clonal DNA contributions) for the contaminant events. 2008 and 2007 Racine contamination data (2008).

**Figure 5.** Adjusted *E. coli* DNA values (expressed as Log CE) versus viable cell counts (expressed as Log MPN/100 mL), Horlick Dam 2007-2008.

**CONCLUSIONS**

With care and consideration, QPCR may be effectively used to monitor water quality in a rapid, efficient manner; providing quicker response time to predictable pollution events. In summary, this Racine, WI cross-comparison study has demonstrated that:

- QPCR, as an analytical method, was capable of adequately characterizing surface waters as part of a routine monitoring program once site-specific profiles were constructed using the USEPA beach sanitary survey tool.

- Application of a corrective factor, employed when ambient background DNA levels were predicted to be high based on a suite of predetermines (ambient indicators, increased flow rate, and precipitation), allowed QPCR to accurately predict beach status and quantity bacterial indicator density within two (E. coli) to four (enterococci) hours of sample collection (Table 1 and 2).

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